Report for 2003NH20B: Pathogens in Biosolids: Evaluation of Clostidium perfringens as an indicator organism to assess the efficiency of biosolid disinfection processes

There are no reported publications resulting from this project.

Report Follows

PATHOGENS IN BIOSOLIDS: EVALUATION OF *CLOSTRIDIUM PERFRINGENS* AS AN INDICATOR ORGANISM TO ASSESS THE EFFICIENCY OF BIOSOLIDS DISINFECTION PROCESSES

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Problems and Research Objectives:

The treatment and disposal of biosolids have been ongoing issues for both public and private wastewater treatment plants. The main objective of many wastewater treatment plants is to find an indicator organism that can be used in processes to evaluate treatment efficiency. This organism should be ubiquitous in fecal material, and not be a public health risk if used in treatment challenges. *Clostridium perfringens*, a non-pathogenic component of all fecal material, has been of interest for some time. By examining the effects of anaerobic digestion on this organism, strong inferences can be made on how the treatment will affect other microbial contaminants found in sewage.

Background Information:

The process of anaerobic digestion can be broken down into two categories based on temperature; mesophilic digestion, which takes place at 35°C, and thermophilic digestion, which takes place at a temperature between 48 and 62°C. The combination of high temperatures and high level of methane produced by the system creates an effective environment for inactivating potentially pathogenic fecal contaminants. The process is fueled by the metabolic by-products of anaerobic organisms, such as *C. perfringens*.

C. perfringens is a gram-positive rod-shaped anaerobe that is part of the normal flora of the human digestive tract. Its most desirable quality, in the interest of this study, is its ability to form environmentally stable endospores. This stability can be compared to the stability of the ova of *A. lumbricoides*, the cysts of *G. lamblia*, and the oocysts of *C. parvum*. *C. parvum* is a small parasite, measuring about 3-5 µm. It lives in the intestinal tract of a variety of animals. The infectious form of this organism is the oocyst, those infected develop Cryptosporidiosis. *G. lamblia* infects humans in the cyst form, causing them to develop a disease known as Giardiasis. It is found all over the northeast U.S. and is commonly related to Beavers. *A. lumbricoides* is one of the largest and most common parasites found in humans. The adult worms live in the small intestine and ova are passed in the feces as the infectious form.

Testing Process:

The testing process can be broken down into the following four phases;

-Recovery of Environmental Strain of *C. perfringens*:

Fresh, raw influent from the Durham Wastewater Treatment Plant was filtered through a small-pore filter by suction filtration. This filter was then applied and incubated on mCP media, selective for *Clostridium* and differential for *C. perfringens*. Colonies were then identified by reverse cAMP testing and identification of double-zone beta hemolysis.

-Spore Induction for *C. perfringens:*

Spore induction for C. perfringens was achieved by inoculating a freshly heat-shocked culture of the bacteria into Modified Duncan-Strong media. Spores were observed under phase contrast microscopy.

-Anaerobic Digestion System:

A two liter dialysis bag was filled with raw influent water spiked known concentrations of *C. parvum*, *G. lamblia*, *A. lumbricoides*, *E. coli*, and *C. perfringens* spores and vegetative cells. Samples were taken from the bag and tested on days 1-4, 7, 11, and 15

-Monitoring the Digestion System:

Spores and vegetative cells of *C. perfringens* were serially diluted and a titer was performed on mCP agar, *E. coli* was assayed according to the EPA approved method for multiple tube fermentation (MPN) technique, *G. lamblia* and *C. parvum* were tested for viability with the DAPI/PI staining method, and *A. lumbricoides viability* was tested by observation for viable helminth ova, microscopically. All results were obtained in duplicate and then converted to percent reduction for each pull.

Conclusions:

The major conclusions made to date include;

>Spore reduction is an indication of the percent reduction of protozoa

>The ova of *A. lumbricoides* remained viable longer than the spores or the protozoa.

>Anaerobic digestion effectively inactivates protozoa and bacterial spores within 4 days

Future Research Objectives:

-Induction of more heat-resistant spores with the addition of activated charcoal to the modified Duncan-Strong sporulation medium.

-Adding MS2 bacteriophage and B40-8 bacteriophage into the anaerobic digestion system and comparing the percent reduction of phage to the percent reduction of spores and protozoa.

-Perform aerobic digestion experiments using an aerobic spore forming bacterium, such as *Bacillus subtilis*, and comparing the effects of digestion on these spores to the effects on *C. parvum*, *A. lumbricoides*, and *G. lamblia*.